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FREE RADICAL-INDUCED CHAIN BREAKAGE IN IRRADIATED AQUEOUS SOLUTIONS OF DNA

G. M. Meaburn
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ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

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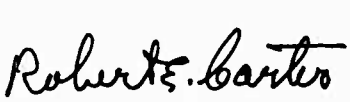

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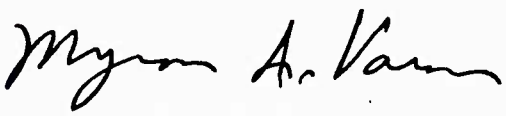

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FOREWORD
(Nontechnical summary)

Deoxyribonucleic acid (DNA) plays a unique role in storing and transmitting genetic information in a living cell. The primary processes of replication and cell metabolism, including synthesis of vital enzymes and other proteins, are highly dependent upon the complex intramolecular organization of DNA. These processes may be seriously modified or impaired if the structural integrity of DNA is destroyed in chemical reactions which follow absorption of ionizing radiation by the cell. Free radicals produced by radiolysis of water surrounding cellular DNA are responsible for much of the initial destruction of the macromolecule. One type of damage found as a consequence of these primary radiation-induced processes is breakage of the polynucleotide chain. The detailed mechanisms of the free radical reactions have not yet been elucidated. It is the purpose of this investigation to obtain a clearer understanding of the nature and extent of those reactions between DNA and water radicals (hydroxyl radicals, solvated electrons and hydrogen atoms) which lead to chain breakage. This aspect of the radiation chemistry of DNA was studied in vitro, using dilute aqueous solutions of calf thymus DNA exposed to both ^{60}Co gamma and high-energy electron radiation. The extent of chain breakage was quantitatively determined in these model systems by measurement of phosphomonoester groups produced at sites along the backbone of the DNA molecule. The report presents information on (1) the relative effectiveness of the different water radicals in producing this type of damage, and (2) the locations of the initial sites of attack in DNA.

ABSTRACT

The extent of chain breakage induced by free radical attack of calf thymus DNA in dilute aqueous solution has been determined by assay of liberated phosphomonoester groups. The relative effectiveness of hydroxyl radicals and hydrated electrons as initiators of this type of damage was investigated in both native and denatured DNA exposed to ^{60}Co gamma and 40 MeV electron radiation in the absence of oxygen. Approximately 8 percent of available OH and 6 percent of e^-_{aq} react to produce chain breaks in double-stranded DNA, whereas these values are reduced to 5 percent and 1-2 percent respectively, for the denatured material. The double helical polynucleotide structure provides protection for reactive sites on the bases which are fully exposed to attacking radicals in single-stranded DNA.

I. INTRODUCTION

Radiation-induced damage to the highly organized DNA structure includes chemical lesions which seriously disrupt the normal functions of a living cell. These include inhibition of mitosis, impairment of the RNA transcription processes and modifications of the reactions leading to protein synthesis.[8] The importance of structural integrity has been discussed by Emmerson[4] in a detailed review of the damaging effects of radiation on the biological function of DNA.

The radiation chemistry of DNA is more readily studied in vitro, in systems which permit detailed examination of the mechanisms and kinetics of the reactions leading to the initial damage. In dilute aqueous solution these changes come about as a result of reactions with products of water radiolysis (mainly OH radicals and hydrated electrons, e^-_{aq}) at specific sites within the DNA molecule. These sites have not yet been completely defined, although much can be inferred about their possible reactivity from experimental data obtained in simpler chemical systems. The extent and type of damage are determined to some degree by the state of the DNA in its aqueous matrix. Intrinsic properties such as molecular weight, intactness of secondary structure and complementary base ratio may significantly influence the course of the free radical reactions. External factors, such as the presence of nucleoprotein, dose and dose rate, and substrate concentration often need to be considered in the interpretation of observable phenomena.

This report deals primarily with breakage of polynucleotide chains in DNA following attack by free radicals. In spite of the numerous measurements made of this induced change in dilute aqueous solution, little information is available on the

mechanisms of free radical reactions responsible for the breaks. The phosphodiester bonds,[2] the C3′-C4′ bond of the deoxyribose moiety,[10] and the loss of a base with subsequent degradation of the sugar[7] have been suggested as sites for chain breakage. A recent study by Kapp and Smith[9] to elucidate the locations and end products of chain breakage by x radiation was carried out in the presence of oxygen. The role of oxygen in the initial processes leading to sugar damage and chain breakage remains obscure at this time. Minimal information relating to mechanisms of chain breakage is available for DNA systems irradiated under anaerobic conditions.

In the present study the extent of chain breakage has been quantitatively determined by measurement of phosphomonoester groups produced in oxygen-free solutions of calf thymus DNA exposed to both ^{60}Co gamma and high-energy electron radiation. The relative effectiveness of e^-_{aq} and OH as initiators of this type of damage has been investigated in both native and denatured DNA. The influence of the secondary structure of the macromolecule on the radiation lability of DNA in aqueous solution is discussed in terms of the nature of the chain breaks.

II. METHODS AND MATERIALS

Two radiation sources were employed in this work, a 22 kCi ^{60}Co facility and an electron linear accelerator (LINAC). Irradiations were carried out at 20-25°C in Pyrex cells of approximately 15-ml capacity. For the gamma irradiations the absorbed dose was determined by Fricke dosimetry over the range 5 to 30 krads using the value $G(Fe^{3+}) = 15.6$ as the number of ferrous ions oxidized per 100 eV input. The dose delivered to each sample was monitored by a 50 cm^3 tissue-equivalent ionization chamber, calibrated to read free-in-air rads at a fixed location in the radiation field.

The dose rate for ^{60}Co irradiation was 400 rads/min. The ferrocyanide dosimeter[12] was used to determine the absorbed dose in solutions irradiated in the electron beam. Single 0.1-μsec pulses of 40 MeV electrons were delivered at 5-sec intervals to accumulate the desired total dose. The dose per electron pulse was approximately 350 rads. The individual pulses were monitored with a Faraday cup located directly behind the target.

Solutions were prepared by adding a weighed quantity of DNA (100 mg/l) to triply distilled water containing Na_2SO_4 to 0.1 ionic strength. Solution was effected by gentle agitation for approximately 18 hours in a shaker bath maintained at 20°C. Denatured DNA was prepared by dissolving weighed quantities of DNA in water without additives, followed by heating to 90°C for 10 min and rapid cooling to ambient temperature in an ice bath. The optical density (OD) at 260 nm was determined for each freshly prepared solution. Values of OD = 1.55 and 2.05 M^{-1} cm^{-1} were obtained consistently for native and denatured DNA respectively, indicating a high degree of double-stranded material in the native preparation and almost complete strand separation in the denatured solution. Furthermore, native solutions showed no signs of deterioration, as reflected by stability of the optical absorption of 260 nm, when kept at room temperature for several days.

The techniques of degassing solutions and filling irradiation cells were similar to those described by Hart.[6] Helium was used to transfer solutions from the degassing vessel to the cells. Nitrous oxide (3×10^{-2} M) was added to a solution as required, after degassing and immediately prior to filling the cells. Tertiary butanol was used as an OH radical scavenger at a concentration of 10^{-2} M. This additive was freshly

distilled in an atmosphere of N_2 and stored under refrigeration until used. Calf thymus DNA (Sigma Type I) was used throughout this work. All other chemicals were of reagent grade. Nitrous oxide was further purified by vacuum line distillation prior to sample preparation. Measurements of pH in the nonbuffered solutions prior to irradiation were found to be in the range of 7.1 ± 0.2. For a single set of samples, however, pH measured as a function of dose fell to a value of 6.4 at 10 krads with no further change up to the maximum dose employed (25 krads). An interpretation of this phenomenon cannot be offered at this time.

Labile phosphate groups produced in irradiated DNA were assayed as inorganic phosphate (phosphate ion) following incubation of samples with acid phosphomonoesterase.[3] Yields of inorganic phosphate were determined colorimetrically by a method similar to that of Berenblum and Chain.[1] Analysis was begun immediately after the irradiation cells were opened and the contents exposed to air. Aliquots of 10 ml were treated with 0.2 M sodium acetate buffer (pH 4.8) and acid phosphomonoesterase (0.1 mg). Following incubation at $37^{\circ}C$ for 48 hours, trichloroacetic acid was added to precipitate DNA which was removed by ultracentrifugation. The supernatant was transferred into a separatory funnel and isobutanol and ammonium molybdate were added. The mixture was shaken and the aqueous layer discarded, followed by two washings with 1 N H_2SO_4. The molybdenum blue color was developed by the addition of $SnCl_2$ solution to the final extract. The alcohol layer and ethanol funnel washings were then transferred into a 10-cm spectrophotometer cell and the optical density measured at 735 nm against an ethanol reference.

A control sample of nonirradiated DNA was included in each set of irradiated samples to provide the "contamination" level of free inorganic phosphate. The difference in optical density between an irradiated and control sample was used to calculate labile phosphate production for a specified dose. Production of inorganic phosphate ions was determined by the same method, excluding the enzyme treatment and the incubation process. A standard curve was prepared using KH_2PO_4 as the source of phosphate ion. Weighed samples of 5′-adenylic acid were assayed and showed a quantitative release of inorganic phosphate after enzyme treatment.

The sensitivity of the method dictates that stringent precautions be taken to prevent phosphate contamination. All glassware used in the analysis was acid washed and well rinsed with distilled water. Irradiation cells were washed with water only and furnaced at 450°C. Reagents were prepared with triply distilled water. The ammonium molybdate solution was prepared by the method of Ging.[5] The tin chloride solution was prepared by diluting 3-ml stock solution (20 g $SnCl_2$/50 ml concentrated HCl) to 100 ml with 1 N H_2SO_4. All reagents were freshly prepared immediately prior to use.

The reagent 2-methylindole was used as a test for malonic dialdehyde. The method of Scherz et al.[14] was employed without modification.

III. RESULTS AND DISCUSSION

Substantial amounts of ionic phosphate were found in irradiated DNA solutions after they had been subjected to the enzyme treatment described above. Phosphate concentration was determined as a function of absorbed dose over the range 1-5 krads. The straight line plots of PO_4^{3-} versus dose shown in Figure 1 are typical of the results obtained for both ^{60}Co gamma and electron irradiated solutions. Radiation

yields, determined from the slopes of these and similar graphs and expressed as G(total phosphate), are summarized in Table I for both native and denatured DNA. Yields obtained in the presence of N_2O, t-BuOH, and air are also tabulated.

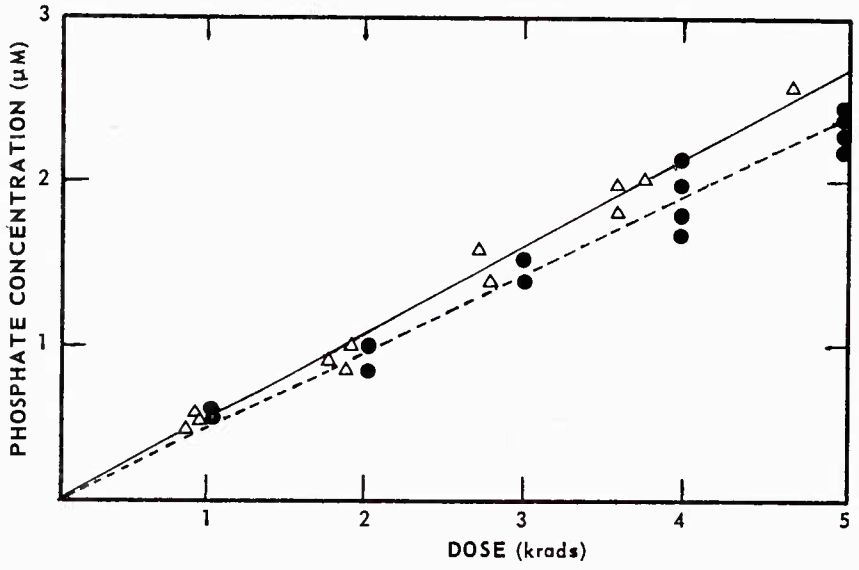


Figure 1. Formation of ionic phosphate (PO_4^{3-}) in irradiated solutions of calf thymus DNA (100 mg/l) after treatment with acid phosphomonoesterase. △ Denatured DNA, 3×10^{-2} M N_2O added, LINAC e^- irradiated, G = 0.52 ± 0.02. • Native DNA, no additive, ^{60}Co γ irradiated, G = 0.46 ± 0.02.

Much smaller quantities of inorganic phosphate were released during radiolysis and were measured without enzymatic release of labile phosphate. The yield of this free phosphate was also directly proportional to dose over the range 5 - 25 krads, as shown in Figure 2 for ^{60}Co gamma and electron irradiated DNA solutions. Phosphomonoester yields, G(PME), were obtained after subtraction of this component from the corresponding G(total phosphate) for each solution. These are shown in the last column of Table I. All yields are the most representative values for a set of observations determined by the method of least squares. The mean errors for each set are also included.

Table I. Phosphomonoester Yields from Irradiated Solutions of Calf Thymus DNA*

Radiation type	State of DNA	Additive+	G(total phosphate)	G(inorganic phosphate)	G(PME)
^{60}Co γ	Native	None	0.46 ± .02	0.044 ± .002	0.42 ± .02
^{60}Co γ	Native	N_2O	0.50 ± .05	0.055 ± .002	0.45 ± .05
^{60}Co γ	Native	t-BuOH	0.16 ± .01	0.019 ± .001	0.14 ± .01
^{60}Co γ	Native	air	0.18 ± .02	-	-
^{60}Co γ	Denatured	None	0.25 ± .02	0.051 ± .006	0.20 ± .02
^{60}Co γ	Denatured	N_2O	0.36 ± .03	0.061 ± .004	0.30 ± .03
^{60}Co γ	Denatured	t-BuOH	0.058 ± .004	0.024 ± .002	0.034 ± .004
^{60}Co γ	Denatured	air	0.17 ± .01	-	-
LINAC e^-	Native	N_2O	0.58 ± .04	0.024 ± .003	0.56 ± .04
LINAC e^-	Denatured	N_2O	0.52 ± .02	0.063 ± .002	0.48 ± .03

* Sigma Type I, at a fixed concentration of 100 mg/l

+ Additive concentrations, $[N_2O] = 3 \times 10^{-2}$ M; $[t\text{-BuOH}] = 10^{-2}$ M; air, saturated

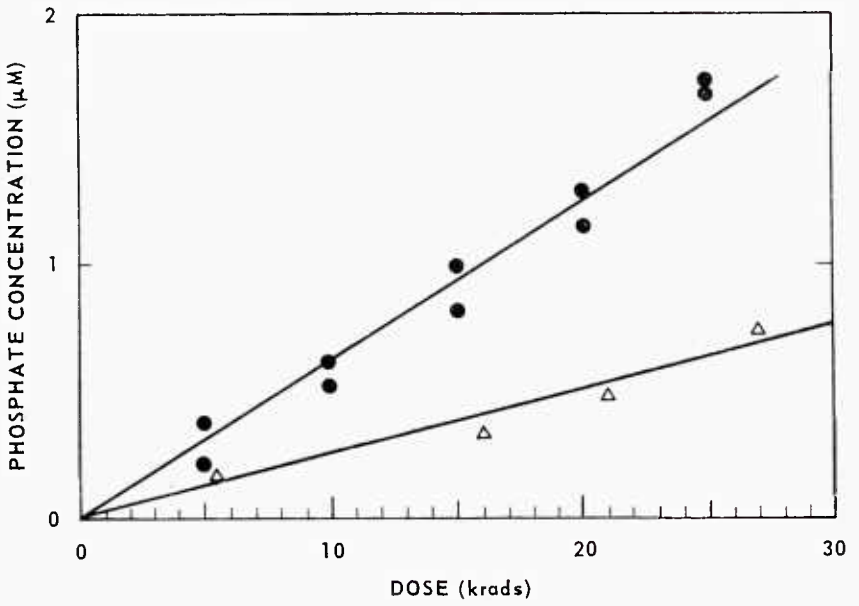


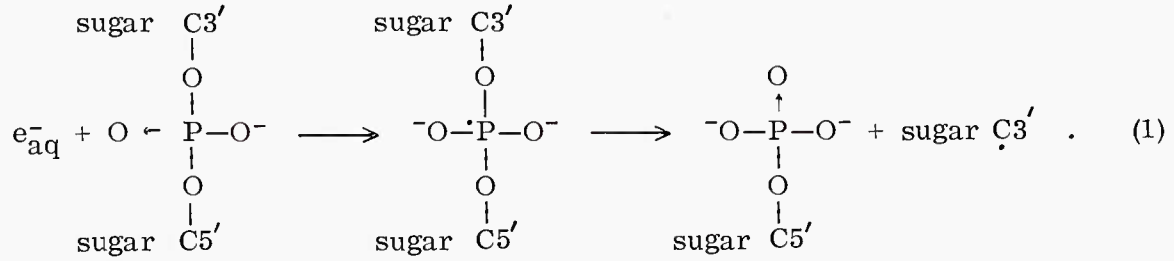
Figure 2. Formation of ionic phosphate (PO_4^{3-}) in irradiated solutions of calf thymus DNA (100 mg/l) without enzyme treatment. △ Native DNA, 3×10^{-2} M N_2O added, LINAC e^- irradiated, G = 0.024 ± 0.003. • Denatured DNA, 3×10^{-2} M N_2O added, ^{60}Co γ irradiated, G = 0.061 ± 0.004.

Solutions containing N_2O were also analyzed for malonic dialdehyde as a radiation product. None was found at doses up to 100 krads, indicating $G < 0.01$ under the prevailing experimental conditions.

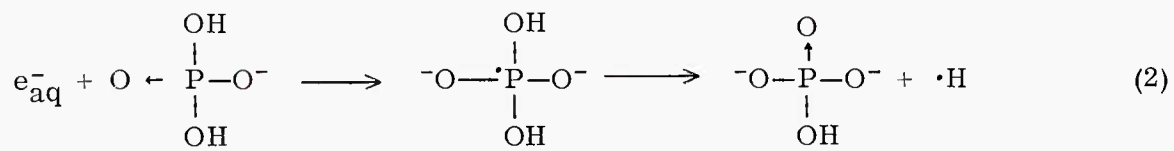
As Kapp and Smith[9] point out, an exact comparison of G values obtained under different experimental conditions cannot be made with confidence. Most of the G(PME) values obtained in the present investigation, for example, were determined for DNA solutions irradiated in the absence of oxygen, in contrast to the conditions applied by several previous investigators.[3,9,13] The role of oxygen in the processes leading to chain breakage is vague and generally not discussed. The DNA concentration used in our work was an order of magnitude less than the lowest employed by Collyns et al.[3] An extrapolation of phosphate yields to 0.01 percent DNA cannot reliably be made from the experimental data reported by these authors, so that a comparison with our results is not possible. An effort has also been made here to keep the absorbed radiation dose as low as possible in order to examine the "initial" stages of radiolysis, an experimental condition not always imposed by investigators. The low dose limit is usually set by the sensitivity and reproducibility of the methods available for analysis of a specific product, in this case phosphate ion. Bearing in mind, therefore, the influence of differing methodology on interpretation of results, we have at this time restricted our aim primarily to one of assessing the relative importance of each of the water radicals, OH, e^-_{aq}, and H, in inducing chain breaks in DNA.

The important yield of phosphomonoester groups in native DNA solutions containing 10^{-2} M t-butanol clearly indicates that the reactions of e^-_{aq} contribute significantly to chain breakage. It is known that e^-_{aq} reacts with the nitrogenous bases in DNA under

similar experimental conditions,[17] a process which might be considered an alternative source of chain breakage.[7] As shown in Table I, however, G(PME) is considerably smaller for denatured DNA with added t-BuOH than for native DNA, even though the bases are more fully exposed to e^-_{aq} attack in the former system. This strongly suggests that reactions of e^-_{aq} with bases do not make a major contribution to phosphomonoester production. A possible mechanism involving attack of the sugar-phosphate moieties of DNA can be visualized.



The formation of a phosphomonoester group at the C3′ position of an adjacent sugar molecule provides an alternative pathway for this reaction. The mode of attack is similar to that proposed to account for the reaction between e^-_{aq} and dihydrogen phosphate producing the oxyanion in a higher state of oxidation.



Pulse radiolysis experiments[18] indicate that the first step in reaction (2) is fast, with a rate constant of 1.5×10^9 M^{-1} sec^{-1}. The sugar radical produced in reaction (1), in the absence of a back reaction, probably undergoes rearrangement, with destruction of its cyclic form and eventual dehydrogenation to satisfy the bonding requirements of the final product.

Hydroxyl radical attack of DNA is responsible for a large fraction of the phosphomonoester yield in both native and denatured material. The reaction probably proceeds by abstraction of an H atom from the sugar moiety, followed by rearrangement of the sugar radical and eventual scission of the polynucleotide chain. Kapp and Smith[9] have discussed the implications of bond rupture occurring at different sites within the deoxyribose structure. Dehydrogenation of the sugar does not necessarily result in extensive fragmentation of this component. This is clearly demonstrated by the formation of negligible amounts of malonic dialdehyde in irradiated solutions of DNA saturated with N_2.[19] Malonic dialdehyde, however, was found as a breakdown product of DNA in oxygenated solution.[9,10] In the present study, deaerated DNA solutions saturated with N_2O showed no detectable amounts of this material.

Nitrous oxide dissolved in aqueous solution is an efficient e^-_{aq} scavenger:

$$e^-_{aq} + N_2O \xrightarrow{(H_2O)} OH + OH^- + N_2 \qquad (3)$$

and approximately doubles the number of OH radicals available for reaction with DNA. Phosphate production is markedly increased by the addition of N_2O to denatured solutions, as shown in Table I. The minimal change observed for native solutions provides additional support for the idea that e^-_{aq}, if available for reaction, induces chain breakage in double-stranded DNA.

There is no a priori reason to suspect that a disproportionately large fraction of the H atoms (themselves representing only approximately 10 percent of the total radical production in neutral solution) is reacting to produce chain breaks. The predominant reaction of H is apparently not dehydrogenation of DNA but addition to the double bond

system of the bases,[15] and there is no evidence that this latter process contributes greatly to chain breakage. As a first approximation it can be assumed that the fraction of H atoms attacking the sugar is close to that of OH radicals reacting with this component of DNA.

It is concluded, therefore, that all three species, $\cdot OH$, e^-_{aq}, and $\cdot H$, contribute to the formation of phosphomonoester end groups. Based on phosphate yields measured in ^{60}Co gamma irradiated solutions and applying the reasoning stated above, G(PME) arising from each free radical source can be estimated. These are shown in Table II.

Table II. Estimated Contribution of $\cdot OH$, e^-_{aq} and $\cdot H$ to Formation of Phosphomonoester Groups in ^{60}Co Gamma Irradiated DNA

State of DNA	Additive	G(PME)	Fraction of G(PME) arising from:		
			$\cdot OH$	e^-_{aq}	$\cdot H$
Native	None	0.42	0.21	0.17	0.04
Native	N_2O	0.45	0.41	0	0.04
Denatured	None	0.20	0.14	0.03	0.03
Denatured	N_2O	0.30	0.27	0	0.03

Scholes et al.[16] have estimated that approximately 20 percent of the radicals from water react with the sugar. Our results indicate that, for DNA in its native state at pH 7, ~ 8 percent of available OH radicals and H atoms and perhaps 6 percent of e^-_{aq} react to produce chain breaks. For denatured material, the corresponding percentages are reduced to 5 percent and 1-2 percent respectively. These figures will be too low if the polynucleotide chain can be broken without formation of a phosphomonoester group, or in a manner in which the phosphomonoesterase does not hydrolyze terminal phosphate groups.

The protective effect of the organized structure of native DNA is apparent for all solutions examined. The reactive sites on the pyrimidine and purine bases, normally shielded to some degree from attacking radicals, are exposed in the single-stranded material. The relative reactivity of double- and single-stranded DNA with OH radicals has been discussed by Loman and Ebert.[11] These authors report that single-stranded DNA reacts at 2.3 times the rate of the double-stranded material. They do not specify the nature of the process. Our results also suggest that the bases of denatured DNA compete more effectively with the sugar moiety for OH radicals. Assuming that damage to the bases does not result in chain breakage, the decrease in G(PME) observed in denatured solutions can be used to estimate the increase in apparent rate of reaction of OH with the bases. A factor of ~1.6 is found, in fair agreement with the quoted literature value.

Recent pulse radiolysis experiments conducted in this laboratory, in which direct measurements of the loss of chromophoric activity were made, indicate for DNA in its native state no loss of hypochromicity within 600 μsec (upper limit of time resolution in these experiments) of the pulse. DNA denatured by heat or damaged by radiation, however, shows severe loss of chromophoric activity following attack by OH radicals within several microseconds of the pulse. These observations are consistent with the hypothesis of Ward and Kuo[20] that the freely diffusing OH radicals cannot penetrate the tightly wound helical structure of DNA. The LINAC e^- irradiations in the present investigation were conducted under conditions similar to those applying in the above-mentioned pulse radiolysis studies, with instantaneous dose rates of $\geq 3 \times 10^9$ rads sec^{-1} being used on both occasions. The decrease in G(PME) observed on changing

from the native to denatured state is considerably smaller than the decrease found in the corresponding ^{60}Co gamma experiments, suggesting that the protection afforded by the helical structure of native DNA is much less than that observed at lower intensities. The explanation of this decreased shielding effect may well lie with the observational time scale of the respective experiments: several hundred microseconds for measurements of chromophoric activity, several hours for determination of phosphomonoester yields. The yields of stable end products are almost certainly influenced by the occurrence of intramolecular reactions involving long-lived DNA radicals. Such reactions would be prevalent at the high dose rates associated with the LINAC e-irradiations.

REFERENCES

1. Berenblum, I. and Chain, E. An improved method for the colorimetric determination of phosphate. Biochem. J. 32:295-298, 1938.

2. Bopp, A. and Hagen, U. End group determination in γ-irradiated DNA. Biochim. Biophys. Acta 209:320-326, 1970.

3. Collyns, B., Okada, S., Scholes, G., Weiss, J. J. and Wheeler, C. M. Chain scission and hydrogen bond breakage on irradiation of DNA. Radiation Res. 25: 526-536, 1965.

4. Emmerson, P. T. X-ray damage to DNA and loss of biological function: effect of sensitizing agents. Advances Radiation Chem. 3:209-270, 1972.

5. Ging, N. S. Extraction method for colorimetric determination of phosphorus in microgram quantities. Anal. Chem. 28:1330-1333, 1956.

6. Hart, E. J. Mechanism of the γ-ray induced oxidation of formic acid in aqueous solution. J. Am. Chem. Soc. 73:68-73, 1951.

7. Hems, G. Chemical effects of ionizing radiation on deoxyribonucleic acid in dilute aqueous solution. Nature 186:710-712, 1960.

8. Kanazir, D. T. Radiation-induced alterations in the structure of deoxyribonucleic acid and their biological consequences. Progr. Nucl. Acid Res. 9:117-222, 1969.

9. Kapp, D. S. and Smith, K. C. Chemical nature of chain breaks produced in DNA by x-irradiation in vitro. Radiation Res. 42:34-49, 1970.

10. Krushinskaya, N. P. and Shal'nov, M. I. Nature of breaks in the DNA chain upon irradiation of aqueous solutions. Radiobiology 7:36-45, 1967.

11. Loman, H. and Ebert, M. Some pulse radiolytic observations on the reactivity of hydroxyl radicals with DNA and poly A. Int. J. Radiation Biol. 13:549-557, 1968.

12. Pagsberg, P., Christensen, H., Rabani, J., Nilsson, G., Fenger, J. and Nielsen, S. O. Far-ultraviolet spectra of hydrogen and hydroxyl radicals from pulse radiolysis of aqueous solutions. Direct measurement of the rate of H+ H. J. Phys. Chem. 73:1029-1038, 1969.

13. Peacocke, A. R. and Preston, B. N. The action of γ-rays on sodium deoxyribonucleate in solution. II. Degradation. Proc. Roy. Soc. London (Biol) 153:90-102, 1960.

14. Scherz, H., Stehlik, G., Bancher, E. and Kaindl, K. 2-Methylindol als Reagens auf Malondialdehyd. Mikrochim. Acta 915-919, 1967.

15. Scholes, G. and Simic, M. Radiolysis of aqueous solutions of DNA and related substances; reaction of hydrogen atoms. Biochim. Biophys. Acta 166:255-258, 1968.

16. Scholes, G., Ward, J. F. and Weiss, J. Mechanism of the radiation-induced degradation of nucleic acids. J. Molec. Biol. 2:379-391, 1960.

17. Schragge, P. C., Michaels, H. B. and Hunt, J. W. Factors affecting the rate of hydrated electron attack on polynucleotides. Radiation Res. 47:598-611, 1971.

18. Thomas, J. K., Gordon, S. and Hart, E. J. The rates of reaction of the hydrated electron in aqueous inorganic solutions. J. Phys. Chem. 68:1524-1527, 1964.

19. Ullrich, M. and Hagen, U. Base liberation and concomitant reactions in irradiated DNA solutions. Int. J. Radiation Biol. 19:507-517, 1971.

20. Ward, J. F. and Kuo, I. The effect of chloride ions on the γ-radiation-induced destruction of DNA, nucleosides and nucleotides in aqueous solution. Int. J. Radiation Biol. 18:381-390, 1970.

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13. ABSTRACT

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